THE APPLICATION OF EXPERIMENT TO THE STUDY OF CANCER

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Dogmatic statements regarding the nature of cancer have broken more reputations than they have made. Paradoxical as it may be, progress in our knowledge of cancer has been made mainly by failure, by successive generations of investigators proving the insufficiency of the knowledge and the hypotheses of their predecessors; but this applies equally to the advancement of knowledge in many fields. The advancement of knowledge by the process of exclusion is surely the most tedious of methods, since, beyond all else, it signifies inability to formulate correctly the problem which it is sought to solve. We are still struggling to formulate correctly the problem, or better, problems of cancer, the sum of our facts not yet sufficing for an unequivocal statement of a problem which shall finally rid us of the everlasting question, What is the cause of cancer? We must be patient of the necessity for undertaking destructive work. In the face of the many defeats efforts at constructive work have suffered in the past, we must be mindful that hypotheses and theories, like men, "may rise on stepping-stones of their dead selves to higher things." Finally the truth is elicited. While avoiding dogmatic statements, I hope to show that the application of experiment to the study of cancer is rational to-day, and has advanced in four or five years to a stage where the results have become of constructive value.

In the space of a short article it is impossible to give an adequate account of the many aspects from which "cancer" requires to be studied. I propose to consider mainly the application of the experimental method to the study of growth, and to show how greatly knowledge has advanced since the first experiment on cancer was made in 1773. At that time

experiment was guided by reasoning from entirely erroneous premisses. For a long time subsequently, knowledge was greatly advanced by descriptive investigations undertaken with the assistance of the microscope. Later, experiment was resorted to again, in applying the methods of bacteriology, with results which showed rather what cancer was not, than what cancer was. Biological knowledge required to advance in other fields before the experimental method could be so applied to the study of cancer as to yield positive contributions, carrying us forward from where clinical, histological, and bacteriological studies had reached their limitations, and from where physiology and chemistry could find no point of attack.

The Academy of Sciences, Belles Lettres, and Arts at Lyons proposed the following as the subject of a prize dissertation for the year 1773: "To form such inquiries on the causes of the cancerous virus as may lead us to ascertain its nature and effects, and best methods of obviating it." The foul sloughy discharge, the "cancerous virus," proceeding from ulcerating, septic, "open cancers," was at that time held to be the characteristic of the disease, and the subject selected was the direct incentive to the first experiment on cancer.

The prize was awarded to Bernard Peyrilhe, who remarks, in the course of his essay, "With respect to the contagious nature of this virus, it must be acknowledged that, either externally or internally applied, it is capable of infecting the healthiest of men." . . . He found that by the subcutaneous injection of what he regarded as the "cancerous virus," he could reproduce those clinical features which were at the time supposed to be characteristic of the disease. The correspondence seemed to him, and to the judges of the prize, to be so close as to merit the inference that he had communicated cancer itself, although the experiment terminated prematurely. His essay was ordered to be published by the Academy. It attracted so much attention that it was translated into various languages, and influenced medical opinion for many years. The experiment really bore on the transference of secondary bacterial infections, and not on the transference of cancer. The experimental investigation of cancer is to-day something entirely different. The *sine quâ non* to its satisfactory pursuit is the fulfilment of the requirements insuring absolute asepsis. The animals which are made to bear cancerous growths artificially, suffer little or no incon-

venience; since secondary bacterial infections are inimical to the objects of the experiments, they are carefully avoided. As will be explained below, the transference of cancer differs fundamentally from the transference of any known infective disease.

Peyrilhe's experiment may, however, be held to have been positive, in that he had produced artificially what were then regarded as the particular features of the "cancerous virus." The repetition of Peyrilhe's experiment to-day could not be justified as a means of throwing light on the nature of cancer. Peyrilhe was under the influence of Descartes' lymphatic theory, as were his contemporaries Alexander Monro and John Hunter in this country. He was ignorant of the true structure both of cancer and of the healthy tissues, and the "cancerous virus" itself was nothing more than the consequences of the action of the organisms of putrefaction on the tissues. It was not till one hundred years later that Lister's experiments on wound infections demonstrated conclusively that this was so.

Progress was about to be made along unsuspected lines, and during a century the valuable additions to knowledge of the nature of cancer remained almost entirely descriptive. The developments of normal and pathological anatomy, of histology and of experimental physiology, riveted attention. They yielded, as the end result, criteria of the minute structure and nature of cancer absolutely different from those by which the success of Peyrilhe's experiment had been tested. The "cancerous virus" survived; but had already acquired a changed and vaguer connotation, when, in 1840, Langenbeck found solid nodules—new formations of tissue—develop in the lungs of animals after inoculating them with cancerous *tissue* from a human breast. Those nodules were taken to imply that the animals had become infected from human cancer, as the nodules in Villemin's experiments (1865) were interpreted—as we now know, correctly—to mean the infection of rabbits by the inoculation of tuberculous matter from the human subject.

The significance of the many resemblances of cancerous to healthy tissues was still unappreciated. It was long in being fully comprehended. Its importance received occasional emphasis early in England, *e.g.* by Pott, Abernethy, Wardrop, Astley Cooper, Walshe, Hodgkin, Everard Home, Carswell, Wilks, and others, and was fully recognised by Johannes Müller and his pupil Virchow. In 1868 Wilks emphasised how this similarity

differentiated the disease from infections. Thiersch, Waldeyer, and others finally established the relation of cancer to normal tissues, and its probable origin from them in many cases, in opposition to the humoral theories which found their last exponent in the distinguished Virchow.

Experiments like Langenbeck's were repeated with great perseverance even up to the present decennium; but, with the development of bacteriology, they were modified in many instances, where the experimenter believed he had discovered the "virus" or other minute parasitic cause of the human tumours. Whereas many observers were unable to induce cancer in animals in those ways, others claimed success. Criticism always effectively disposed of those claims. The tumours produced in animals bore vague, or even only imaginary, resemblances to the tissues of the human tumours employed in the experiments. Many were proliferations of the inflamed tissues of the animals themselves, as the result of the experimental inoculations, while others were normal and pathological structures encountered in animals, but not in man, and entirely independent of the experiments.

All this time descriptive pathology and study at the bedside were advancing knowledge, leading ultimately to the accumulation of an overwhelming amount of evidence in favour of conclusions in two directions: (1) the cancerous tissues destroyed life mainly, if not solely, by their powers of progressive proliferation and of dissemination in the body: (2) the proliferation arose primarily in a circumscribed area, and proceeded from causes inherent in the growing cells themselves. It required many years before the early surgical treatment of the disease ousted all other methods, and was acknowledged to be justified absolutely by increasing certainty of the circumscribed origin of cancer. This had been clearly enunciated by Wilks as a result of investigations pursued between 1847 and 1868, and was stoutly defended by Campbell de Morgan and Charles Moore, against the constitutional views of the disease ably expounded by Paget and his followers. The opposing schools joined issue in a memorable debate before the Pathological Society of London in 1874. The methods of descriptive pathology and of clinical study ultimately reached their limitations, for they were inadequate to explain the powers of growth, which they had sufficed to

define as embodying the essential problem of cancer. So distinguished an observer as Campbell de Morgan confessed that "experiment fails us, and we know nothing of the earliest stages of the disease." The search for a specific cancer cell proved futile, according to Wilks, as early as 1868. Investigation of the minute intra-cellular structures provoked much controversy during the last twenty years of the nineteenth century, but remained, as it remains to-day, barren of positive results. Discussion of the infective or non-infective nature of cancer became entirely hypothetical. All attempts to induce cancer in animals by inoculation from the human subject having led to negative results, the method had been practically abandoned. Efforts to elicit experimentally the hypothetical (latent) powers of growth of embryonic tissue in support of Cohnheim's hypothesis, or those of groups of adult cells whose "organic continuity" with the tissues was dissolved, as postulated by Ribbert, showed how limited were the powers of growth of embryonic and adult tissue. In the case of embryonic tissues they showed also that the ultimate cessation of growth was accompanied by a differentiation into tissue of adult type.

The acquisition of new knowledge seemed very remote indeed. In spite of the most discouraging outlook, courageous and serious resort to experiment had become a pressing necessity at the end of the nineteenth century, and, fortunately, the success attending subsequent experiments has conferred a positive value on much of the apparently negative experimentation described above.

The study of the phenomena called forth by inoculating the tissues of animals into others of the same, and of alien species, showed during the closing years of the last century how distinct —chemically and biologically—the tissues of one species of animal are from those of another, even when nearly allied and elaborating their tissues from identical pabulum. The demonstration of the difference appears to have re-directed attention to a few earlier experiments on the transmission of tumours from one animal to others of the same species. Statements of the success attending the deliberate inoculation of human beings with portions of their own cancerous tumours were remembered. Observations made by Hanau, Jenny, Morau were remembered as indicating the transference of the disease from rats and mice to others of the same species.

It was by no means clear how those experiments were to be

interpreted, nor how the disease had been transmitted, and they had been received with scepticism apparently justified by repeated failures to transmit human cancer to animals. The recollection of them was born of the despairing hope that, perhaps, after all, statements relating to cancer in animals had been unduly neglected. It had been asserted frequently that the disease was peculiar to man, even to civilised man, that it was rare in other races of mankind, and in the domesticated animals with whom he came in contact, but altogether absent in aboriginal races and in wild animals. It is only fair to add in passing that veterinarians had described all the main types of cancer in the domesticated mammals.

At the time of the foundation of the Imperial Cancer Research Fund the position was briefly as follows: The gradual exhaustion of the methods in vogue had led to a cessation in the advancement of knowledge on the nature of malignant new growths. The bedside observation of cases in the human subject, combined with anatomical, microscopical, and chemical examinations in laboratories attached to hospitals, had advanced to a stage where they seemed to raise more difficulties than they solved. As in the corresponding stages of previous attempts to solve other anatomical and physiological problems, the number of contradictory speculations current was legion. They were based on the same observed facts, which were being added to continually by other facts of the same kind—*i.e.* there was vain repetition. The exact knowledge of the disease was strangely restricted to its occurrence in the more civilised races of man, while the explanations of the observed facts roamed over the full field of medical and biological knowledge, and speculation. This discrepancy required adjusting. The impossibility of directing speculation into proper channels, or even of determining what those channels might be, by other methods, caused the need for comparative studies and for experiment to be acutely felt.

The first organised attempt to broaden the basis of the knowledge of cancer, and of its experimental study, was made when the investigations of the Imperial Cancer Research Fund [1]

[1] A "Society for Investigating the Nature of Cancer" was formed at John Hunter's instigation in 1804. The German Cancer Committee was founded in 1900; its work was, however, directed to applying statistical methods to prove the infective nature of cancer. The New York State Cancer Laboratory at Buffalo was in working order in 1899, the work has been almost entirely experimental; but mainly directed to search for evidence in favour of infection.

took definite shape on the basis of a scheme of research submitted to the Executive Committee by myself in October, 1902, and diligent search was instituted into the occurrence of cancer not only in all races of mankind, civilised and aboriginal, but also throughout the animal kingdom. A farm was secured for observations on the larger domesticated animals suffering naturally from cancer, and every opportunity was utilised of testing the possibility of transferring the disease and studying it under experimental conditions. The scheme of experimental research, thus embarked on, received an unexpected justification and a great impetus by the publication of Jensen's accurate and fundamental observations on the inoculation of a tumour of a mouse in 1903, and contemporaneously of those of Borrel. Since then valuable work has also been done by Apolant, Haaland, Loeb, Michaelis, Henke, and, above all, by Ehrlich.

Jensen's great service consists in the fact that he did not restrict himself to demonstrating the reproduction at the site of inoculation of the features of the original growth. He also proved,[1] by carefully following the processes at the site of inoculation step by step, that the new formation was due to the continued growth of the cells peculiar to the tumour, parts of which he had introduced. Jensen's work has been energetically followed up in all civilised countries, and in the first instance in the laboratory of the Imperial Cancer Research Fund.

The investigations of Murray and myself on an adenocarcinoma mammæ and Jensen's own tumour confirmed his observations, and, by extending them to a large number of other malignant new growths in mice, established the experimental study of cancer on a secure foundation; so that, after some preliminary controversy, it is now universally agreed, infection plays no part in the process of experimental transference. On the contrary, transference to fresh mice merely affords the cancer cell an opportunity for continuing to proliferate in fresh soil. With Murray I made an exhaustive study of the processes at the site of the inoculation of cancerous tissue in the case of

[1] This demonstration is sometimes erroneously attributed to L. Loeb, who obtained tumours on inoculating sarcoma in rats in 1901-3. Loeb's original description of the processes at the site of inoculation does not bear this interpretation. He also admitted the possibility of the tissues surrounding the grafts acquiring sarcomatous properties.

twenty sporadic mouse tumours, and conclusively proved that the cancer cells are merely transplanted into the new animals which provide them with nourishment, by means of a specific connective tissue reaction supplied afresh after each fresh transplantation.

The criticism, that the tumours experimented with were not comparable with cancer in the human subject, was advanced as speedily as of yore, and with all the assurance born of a knowledge of its effectiveness in discrediting earlier experiments. It is still feebly uttered by certain persons who lack extensive experience of cancer in animals. At last, however, it was to be met satisfactorily. Before proceeding to the study of other problems, Murray and myself spent much time in demonstrating, not only that the starting-point of the experiments has been cancer of the mamma, but were at great pains to reproduce artificially metastases, infiltrative and expansive growth—in short, all the main features of the disease in previously healthy animals. With Cramer we drew a parallel between the growth of cancer under natural and experimental conditions. Haaland independently did the same for a number of carcinomata, and demonstrated that Ehrlich's experimental sarcoma formed metastases, and behaved in other ways like a malignant new growth of the human subject. Apolant published an elaborate and valuable monograph on the histology and pathology of the mammary tumours of the mouse.

These results are based on observations all but limited to the mouse. As yet our numerous inoculations of cancer in other vertebrates have met with only transitory success in rats and in a single carcinoma mammæ of the dog. Even in the case of mice there are great difficulties to be overcome: for the other vertebrates many of the difficulties are still unsurmounted. The failures may be due largely to the relatively small number of inoculations made as contrasted with mice, as well as to difficulties associated with varying suitability of the animals employed, which the study of the transplantation of cancer in mice has proved is a factor of great importance.

The inoculation of cancer in mice has been necessary on a scale perhaps unprecedented in the experimental study of disease, in order to prove that the cancer cell is able to grow and to proliferate continuously in animals of the same species only. To establish that the cancer cell is not transferred

naturally from one individual to another, equally extensive observations have been necessary. As I shall show later, the cancer cell of the mouse is in all probability a mouse cell incapable of nourishing itself and proliferating, except when in intimate organic union with the body, and nutrient supply, of the living mouse. Ehrlich has shown that a transitory growth follows the transplantation of one of his mouse tumours into rats. We found that the same power is possessed by other mouse tumours. Growth, however, speedily ceases in rats, although it is retained unimpaired if the cells be re-transferred to mice after a short sojourn of six to eight days in the rat's body. A mouse tumour does not grow equally well in all mice. We found difficulty at first in getting Jensen's Danish tumour to grow in English mice, and Michaelis and others have entirely failed to get it to grow in the mice of Germany, England, and other countries.

The progress made by the demonstration, that cancer could be transplanted and artificially propagated *ad libitum*, was not of the obvious kind appealing to the multitude. If transference had proved that the tissues of the new animal acquired cancerous properties, *i.e.* that the disease could be conveyed by way of infection, our knowledge of its nature would have been advanced enormously at one step. Then investigation could have been legitimately limited at once to identification of the agent and of the channels of infection.

The nature of the transference of cancer was out of accord with all known processes of infection, in this respect merely presenting old problems in a new light, as well as new problems for solution. However, Murray and myself were able to draw certain conclusions other than those drawn by Jensen, which have been abundantly confirmed since by others: (1) The amount of proliferation exhibited is enormous, once the primary difficulty of transplanting a mouse tumour at all has been overcome. The proliferation is then out of all conformity with the laws of growth in vertebrate organisms. The cancerous tissue retains its histological characters. (2) If a large number of healthy mice be considered, growth proceeds readily in mice in which cancer is rare naturally. Although the maximum incidence of cancer occurs in the later years of life, growth proceeds as well, and even better, in young animals than in old. Therefore, if senescence is intimately bound up with the

origin of cancer, it is unnecessary for growth to continue. (3) The conditions of the origin of cancer were to be sharply distinguished from those of continued growth. (4) The progressive, apparently vegetative, growth was to be explained as inherent in the cancer cells without the assumption of the stimulus of extraneous agents. The amount of growth presented a cell problem of importance in itself and required to be further analysed. (5) The purely cell problems of cancer required to be attacked by the methods of experimental and comparative biology rather than from the narrower standpoint of human pathology.

Those conclusions would have been illegitimate if drawn only from experiment at that time. They were advanced also on the basis of a comparative study of the disease in the vertebrates down to marine teleostean fishes living in a state of nature, and after careful statistical studies of the national mortality from cancer, and of its occurrence in the patients of London hospitals. The zoological distribution of cancer made it obvious that it developed in man, independently of any direct influence proceeding from civilisation, diet, or other one of many inconstant external factors. Subsequent studies in the same direction, and also into the ethnological distribution of the disease, have but rendered this inference more justified. The disease has been found in all those races of mankind among whom diligent search has been made. Where it was said to be rare it has been found to be common—*e.g.* in Japan some 25,000 deaths are annually recorded from cancer. Where it was said to be absent it certainly occurs. Of course, no comparison or statement of relative frequency is possible between civilised and aboriginal man, and between tame and wild animals. The fact of prime importance is that the disease is of universal occurrence, pervading all forms of vertebrate life, and everywhere adapting itself to the conditions presented by different species. The disease is universal, but the difficulty of transferring it even to individuals of the same species is unique, and when successful is not to be confounded with the production of cancer *ab initio*.

Throughout the entire distribution of the disease two facts stand out prominently: The number of cases recorded is in direct proportion to the care with which it is possible to make examinations, and to the number of adult and aged individuals

examined. That cancer develops with increasing frequency with advancing years in all animals, whether their lives be long as in the case of man, or limited to two or three years as in the case of the mouse, is a biological law of universal application. The only class in which cancer has not yet been found, viz. the reptilia, is the exception proving the rule, for in them life may extend to hundreds of years, and the opportunity for examining a sufficiently large number of aged reptiles does not exist. The same applies, if in lesser degree, to the apparent rarity of the disease in wild animals generally, since they do not naturally survive the functional activity of their teeth or their reproductive system.

The greater recorded frequency of cancer in domesticated mammals as compared with wild animals implies, not that the disease is communicated from man, but simply, that man protects them and provides for them, so as to permit of their attaining their respective cancer ages.

In the case of the organs of the human body, cancer exhibits a corresponding dependence on the duration of life, *e.g.* chorion epithelioma develops before birth and presents a perfect parallel to the incidence of cancer in short-lived animals. In a general way it may be said, since space prevents my discussing details, other organs present all gradations up to the skin, which is functional so long as life lasts, and in which the maximum incidence of cancer is only attained in extreme old age. The age of the individual organism is of less moment than the senescence of its several tissues in determining the incidence of cancer. Hence cancer may occur at any age, in the new-born, and even before birth. As a matter of fact it is more frequent at birth than between the tenth and fifteenth years of life. It is therefore wrong to assert that cancer is caused by "old age."

The generalisation as to the constant association of the incidence of cancer with senescence adds greatly to the significance of its unbounded proliferation, when propagated by transference from one set of mice to another *ad infinitum*, since the size of the body and of its individual organs, as well as the length of life, are specific for each species of animal. They distinguish different species as sharply as any of the other criteria on which the systematic zoologist depends for his classification, *e.g.* as sharply between the mouse and the rat as between the mouse and the elephant. As I shall describe below,

cancer frequently stops growing, and this is the most encouraging result obtained by experiment. Nevertheless, there is as yet little reason to suppose that limits corresponding to those set to the growth of organisms and their organs, are necessarily set to the growth of cancer in any of the vertebrates, except when artificially set by the death of the animal in which it originates or is growing. The primary transplantations of our sporadic tumours have succeeded for 66 per cent., and ultimately all may be found to be transplantable. Hence, since a mouse tumour has now been growing twice as long as a mouse lives, the more limited evidence of a similar phenomenon in other vertebrates, *e.g.* rats and dogs, is probably due mainly to insufficient experimentation, and to failure to supply suitable soil. On the one hand, we have the limited powers of proliferation of normal tissues which never produce tumours when transplanted, and the unlimited proliferation of cancerous tissue; on the other hand, we have the dependence of the inception of cancerous change on senescence of the tissues as determined by the laws limiting the duration of life specifically. We have stated that in this association lies the crux of the problem of cancer, and R. Hertwig in referring to physiological senescence, and others, have also noted its biological importance. This association involves a problem which cannot be attacked directly at present, because we are still unable to determine the inception of cancer experimentally. We can, however, approach it indirectly by the prolonged study of the growth of cancer when once it has started, and endeavour to determine if it be purely vegetative, dependent entirely on the supply of adequate and suitable food, or be perhaps also maintained and renewed by some intra-cellular reorganisation occurring periodically, at a time when growth tends to cease naturally. In short, we must find out if the study of growth gives us any clue to its nature and origin. We can also study the conditions favouring and retarding its continuation or modifying it. Thus, the experimental study of cancer is at present essentially the experimental study of the growth of cancer; but this is no subsidiary attribute—it is in reality that property of cancer by virtue of which it destroys human life. It is customary to consider the growth of tumours as expansive or "benign," and infiltrative or "malignant," a classification corresponding also to their clinical behaviour. As a matter of fact

the two forms of growth are interchangeable experimentally. For different carcinomata Murray, Cramer, and myself have shown that this interchange of the mode of growth is dependent on the anatomical surroundings of the tumour, and not on an alteration of the properties inherent in the tumour cells. It therefore suffices to study mere growth, and in doing so one may disregard the features of most use in the current classification of tumours.

The transplantation of a sporadic tumour is effected according to our method as follows: The tumour is removed from the animal so as to avoid bacterial contamination, and weighed. It is divided into portions which are placed in separate receptacles. The portions are then inoculated by means of hypodermic needles, minute fragments (*circa* 0·01—0·03 grm.) being broken off and inserted subcutaneously. The tumour is thus distributed over as large a number of mice as possible—it may be fifty or four hundred. The experimenter must then possess his soul in patience. Rarely is he rewarded by the appearance of true tumours, at the site of inoculation, within a fortnight. He may have to wait from three to six months before the inoculations can be pronounced to have fallen out either positively or negatively.

The nature of the result is determined mainly by two factors: variations in the suitability of the soil the mice afford for the growth of the grafts introduced as above; and variations in the character of the tumour cells, not only of different tumours, but of one and the same tumour. The suitability of the soil may be taken to be fairly constant when large numbers of young mice of the same age (six to eight weeks) and of the same stock are employed. Adult and old mice are not only much less suitable than young animals, but they also exhibit greater individual idiosyncrasies. Normal mice may be said to offer a certain unsuitability, or to be resistant, to inoculation, when tumour cells are transferred to them after removal from their natural environment in the animal in which they developed. This resistance acts as a sieve, sifting out the tumour cells which are unable to nourish themselves and proliferate in this new and, it may be, very strange environment. Thus the positive results of the primary transplantation seldom form a high proportion of the animals inoculated. They are probably obtained by the segregation of groups of cells of high assimilative energy and

growth, in mice whose resistance is below the average, or rather the suitability of whose soil is above the average. A summary of some observations we have already published will serve as an illustration. The final results of transplanting 32 spontaneous tumours of the mamma show that 2,278 of the mice inoculated remained alive three weeks later, and 72 of them ultimately developed tumours—*i.e.* only 1 inoculation out of 31 was successful, or 3·2 per cent. A tumour is propagated by a repetition of this process of transplantation from one series of mice to another. But the subsequent transplantations are only exceptionally accompanied by better success. In illustration of the pertinacity necessary in such experiments, I may state that it was not till we had repeated the primary process above described for twenty-seven different sporadic tumours, and also repeated it for each tumour one, two, three, and four or more times, that we alighted on a growth capable of unlimited propagation. This difficulty in starting propagation illustrates merely one aspect of the initial obstacles which those who embarked on the experimental study of cancer have had to overcome in the case of mice, and are still striving to overcome in the case of other animals.

When once a tumour has been got to grow well in mice of a particular race, similar difficulties are often encountered in transferring it to mice of strange race. Thus we added the study of Jensen's tumour, which was accustomed to Danish mice, to those discovered by ourselves with the greatest difficulty. Michaelis and other investigators have failed altogether to get Jensen's tumour to grow in strange mice. We failed also to propagate a tumour sent from Paris by Borrel. Our experience leads me to surmise that those absolute failures may have been due partly to an unfavourable phase of growth of the cells of the tumours. Our initial success with Jensen's tumour was only $4\frac{1}{2}$ per cent. of the inoculations.[1] When the tumours grown in English mice were transplanted again into English mice, the percentage soon equalled that obtained by Jensen in Danish mice, and in single experiments greatly exceeded it,

[1] The tumour had been removed from a mouse, was forwarded by ordinary post hermetically sealed in a glass tube, and transplanted into English mice five days later. Our primary transplantations were therefore made with an added difficulty, but they also illustrated the vitality of the tumour cells after separation from the body.

rising to 90 per cent. at the third transference. When once the initial difficulties have been overcome, the growth exhibited by propagated cancer, as illustrated by several of the tumours we have studied, soon passes beyond the bounds of all measurement. A small piece of tissue weighing no more than 0·01 grm. frequently increases to 1·5 grm. of tissue in ten days. This is an increase of over a hundredfold, and under suitable conditions 90 to 100 per cent. of the animals develop tumours through many successive transplantations. This sequence of events is not constant when tumours can be transplanted successfully.

Some sporadic growths grow well from the outset of propagation. For others a marked increase in the success of transplantation is observed, frequently after three or four transferences. For others again the percentage of successful inoculations cannot be raised to, or maintained at, 100 per cent.; but remains constant within certain fluctuations. However, for the majority of our carcinomata growth ceased at the third or fourth transference. We have interpreted those differences to signify primary differences in degree, inherent in the cells of different tumours. They persist during continued propagation in the majority of cases; so that each tumour exhibits from the outset of propagation a constant behaviour within limits. We have refrained from terming the increased success of transplantation occurring in special cases "an increase of virulence," as has been done by analogy with what occurs when bacteria are propagated by the method of *passage* from one animal to another. The term "virulence" should be dropped and "adaptability" substituted, because the method of propagation is essentially an artificial selection of the most rapidly growing tumours, and their subdivision and distribution over a large number of animals. Although the greater success sometimes attending later inoculations suggests an alteration in the energy and rate of growth of the tumour cells, the phenomenon referred to may be explained without having recourse to this assumption: (1) by an increase in the number of proliferating cells, similar to what occurs in the propagation of domesticated animals as in the following example; and (2) by adaptation.

The increase in the numbers of any form of fancy dog which happens to become fashionable is due not to an artificial shortening of the period of canine gestation, but to increased breeding facilities, *e.g.* to multiplication of the number of those

who find it profitable to breed that kind of dog. There is a multiplication of the number of dogs breeding, so in the artificial propagation of cancer we multiply the number of cells proliferating. I discuss later the possibility of biological alterations leading to increased rapidity of cell growth. Propagation segregates in the first instance the cells which can accommodate themselves to the artificial conditions, and then provides for their nutrition and proliferation. Thus there are more of the suitable cells in each of the later grafts, and within a given period, this primary advantage gives bigger tumours from each single graft, and a higher percentage of tumours over the total number of animals inoculated.

A violent change in environment is effected when a tumour is removed from the animal in which it developed. The manner in which the change is effected is important. Whereas we have succeeded in transplanting 66 per cent. of the sporadic growths we have discovered, Ehrlich has transplanted 14 per cent. of his tumours successfully. The discrepancy is certainly due to the difference in method. As a rule only the tumours best suited to propagation survive Ehrlich's procedure, while we have obtained a broader basis for the study of the growth of tumours, since our material includes tumours of all degrees of transplantability. One group of tumours does not survive the process at all. A second group grows for a variable time and then dies, being unable to adapt themselves to a strange and presumably an unfavourable environment. The tumours of a third group are able to adapt themselves gradually. The fourth group adapt themselves quickly or at once, and this is the small group which are easily propagated. It is possible that among them there are tumours surviving transplantation, not only because of the inherent properties of their cells, and their capacity for adaptation, but because the change in environment is in reality to a more favourable soil. In the third and fourth groups there must be also more and more cells suited to propagation in the grafts at each additional transference, till a maximum is reached. Although the group of tumours giving maximal success on propagation are of practical importance in experiment, because of the ease with which they permit of controls to attempts to modify growth, their theoretical value depends on their relation to the varied degrees of energy of growth exhibited by the whole group of tumours. It is

obviously of importance to determine experimentally whether or not all tumours tend to augment their energy of growth to the maximum exhibited by this group, and at the same time to assume the same histological garb. At present many tumours appear to retain their own peculiarities, and although the energy of growth may increase, it is not yet possible to determine whether or not the tumours of one organ ultimately approximate to one form when growth is prolonged sufficiently.

I may point out here that the first and second groups of tumours behave on transplantation as embryonic and adult tissues do, by exhibiting limited powers of proliferation, with the difference between cancer and embryonic tissue, that the former does not differentiate into an adult form. We are therefore able to place a positive value on the negative efforts which have been made to produce tumours by propagating normal tissues, and we are able to establish all gradations from the limited growth of normal tissues under experimental conditions, up to the remarkable phenomenon exhibited by the unlimited growth of the cells of some cancers under the same conditions. Thus experiment has bridged over the gulf between normal and cancerous tissue so far as their powers of growth are concerned. The limited growth of normal tissues, when transplanted, is independent of any extraneous organism. There is no need to assume such a dependence when cancerous tissues exhibit corresponding limitations; and if not, why should it be necessary to assume this intervention when the powers of growth pass insensibly through all gradations to those of unlimited amount?

The possibility of an accelerated *rate* of proliferation may be entertained when a tumour has been continually transplanted during long periods, as in the case of Jensen's tumour. If I understand Ehrlich rightly, this is what he means when he states that the increase in "virulence" is due to the avidity of the receptors having attained a maximum in consequence of rapid passage from animal to animal. I shall show immediately that there are natural fluctuations in the amount of proliferation. In the case of rapidly growing tumours all the inoculations which are successful yield tumours quickly (within, say, ten days). Mice then negative remain so. The fact shows that rapidity of proliferation is closely bound up with an extreme susceptibility to nutritive requirements. Since growth presents

natural variations, an increase in its rate may be one result of our artificial selection in long-continued propagation. In passing I may merely mention it is now well established that the amount of proliferation can be influenced in the opposite direction—viz. diminished by exposure of a tumour to heat. The environment may be of great importance when a tumour is propagated for years. Theoretically the long-continued growth of cancer cells in the soil provided by the mice of one stock or country may handicap them for growth in the different soil provided by mice of another; but, whether this be so or not, augmented adaptation to an accustomed soil may result in an enormously increased proliferation. In this direction therefore the cells of later generations may be biologically very different from those remotely antecedent. What occurs when a tumour is propagated by transference through many generations of mice is determined not so much by the number of transferences, as by the long duration of the particular environment, breeding as it were a cell of particular quality. If one follow the process of artificial propagation back step by step to the primary animal, there is no reason why one should stop there, since the gulf between the growth of cancerous tissue and normal tissue after transplantation has been bridged over. What takes place in artificial propagation may well be but an artificial reproduction of what had long been going on naturally in the animal in which cancer developed. However, it is inadvisable to pursue such speculations further at present, for the conditions of the growth of cancer in the spontaneously attacked may be different from those in normal animals, although the lesions are the same. At any rate, the differences between individuals may be of prime importance in determining the nature of growth in the primary transplantation as well as in the individual spontaneously attacked. It seems, at least, that the preceding considerations give us a deeper insight into the nature and clinical behaviour of the cancers of man during their continued growth, and when they exhibit what appears to be a change to a more malignant condition.

The phenomena of growth can all be explained without the assumption of microbic interference. And this brings me to the further consideration of some matters of clinical importance. When the growth of a tumour under artificial propagation is

contemplated as a whole, fluctuations in the rate and amount of growth are observed, even when many batches of the inoculations yield 90 to 100 per cent. of tumours. These fluctuations can be considered from two standpoints: either they are due to variations in the suitability of the mice inoculated, or to others inherent in the tumour cells. While variations in the suitability of the mice, or even of the same mouse at different times, no doubt contribute to their production, they are insufficient to explain all the phenomena. The fluctuations may be revealed by (1) variations in the success of transplantation, (2) a contrast between the sizes of the tumours in different batches, or even in the same batch, (3) a tumour which is growing rapidly may come to a standstill, diminish in size, subsequently grow rapidly, and again diminish, (4) diminution in size, however, is frequently followed by entire disappearance and absorption of the tumour. Those features of growth can be studied in parallel experiments in such a way as to bring out the probability that they are for the most part manifestations of a variation in the assimilative energy of the tumour cells themselves. In the human subject there are corresponding fluctuations in the growth of cancer. In one part of a tumour growth is proceeding rapidly, in another part slowly. Periods of exacerbation alternate with periods of amelioration. Further, secondary nodules of growth are known to disappear while others are growing, and occasionally primary growths have disappeared. The observations made on mice emphasise the importance of those clinical features, largely discredited, in the human subject, since they have been often interpreted to mean that the growths exhibiting them were not cancer. The experimental propagation of cancer has demonstrated conclusively that mice can recover from artificial tumours. In the elucidation of this process, and of its counterpart in spontaneous cancer, lies the hope of assisting the surgeon. At present I can only foresee a still remote possibility that dissemination from the primary focus may be hindered; I can foresee no probability that the primary focus will be got rid of by other means than early surgical removal.

The ultimate absorption of transplanted tumours requires to be considered in relation to certain other facts. The mice in which it has taken place are protected highly against subsequent inoculation with the same growth, and to a lesser extent against

other growths. There is thus a degree of protection which is common, and a certain degree which is specific. The spontaneous disappearance of tumours occurs, entirely beyond our control as yet. But the protection it confers can be imitated artificially. The same degree of common protection can be conferred by the preceding inoculation of tumour material if followed by no growth, *i.e.* if absorbed, and this is not so remarkable as the fact that it can be induced also by the injection of the normal tissues of the mouse and most readily by normal blood ; but not by the cancers or tissues of alien species. The far-reaching significance of the protection which can be induced by carcinomatous, by sarcomatous, or by normal tissues, causes me to emphasise that our observations and Ehrlich's are in essential agreement. We require to learn whether the protection thus conferred is actively induced or only passively conferred. In the case of blood this question was most easily settled. The protection is not passively conferred by the serum, but is actively induced by the blood cells. To sum up, we are able to so modify the soil provided by mice that cancer cannot grow in it. For indications of the change we are dependent on the behaviour of the living cancer cell in the living mouse; we are dependent on experiment absolutely.

Jensen recorded the disappearance of tumours in 1902-3. The first occasion on which we noticed a large transplanted tumour disappear was while studying a batch of mice with his tumour in August 1904. This tumour was exhaustively studied, and the important part phagocytosis played in the process discovered, not only in the case of this entire tumour, but in more limited areas of a very large number of tumours. We have since recorded the same process for other carcinomata, and its importance has been emphasised by others, especially by Clowes. We have observed the same process in localised areas of spontaneous tumours. There can therefore be no doubt that the animal primarily attacked attempts to protect itself by the same means as a normal animal after successful inoculation.

The investigation of the nature of protection is bound up with very great difficulties, which are not diminished by the absence of any analogy with what we know of immunity to infective diseases. Up to the present I have spoken of the propagation of the tumours as if it meant a mere culture *in vivo* of the cancer cells. This, however, is only part of the truth.

As I pointed out on a previous page, the cancer cells acquire at each transference a specific stroma, the connective tissue reaction they call forth in the host. A culture of isolated cells has not been obtained, since growth always proceeds in the form of a tissue with intimate vascular supply. We emphasised the distinction thus revealed between the cells of different growths nearly allied histogenetically, and the parallel it presented to the biological reactions whereby blood relationship has been established. At the same time we pointed out its far greater delicacy. In what I have briefly outlined with regard to protection the same delicacy, or specificity, appears. The cancer cell is dependent on the provision by the host of a connective tissue and vascular reaction, if it is to grow into a tumour. It would appear to be a piece of short-sighted generosity on the part of the host to supply connective tissues to subserve merely the needs of the greedy cancer cells, and yet this appears to be the relation which subsists when a tumour is growing well. The question arises, is the connective tissue reaction supplied only in response to the needs of the cancer cells, or is it really protective, as in the case of the similar reactions elicited by the tubercle bacillus or other organisms? When a tumour undergoes spontaneous absorption, the connective tissue reaction does become protective, and the acquisition of phagocytic properties by its cellular elements is the main factor in removing the remains of the tumour. Thus under certain conditions the organism is certainly capable of defending itself very actively through reaction to the presence of the cancer cells, and after the process remains protected. The conclusion that phagocytosis is the only agency by which protection is conferred would in my opinion be unwarranted at present. Spontaneous absorption appears to be frequently associated with a phase of depressed growth of the tumour cells themselves. We do not yet know exactly whether the cancer cells require to undergo changes—either spontaneous, or induced from the side of the host—placing them at times at the mercy of the connective tissue which, at other times, appears to be their willing servant. When a graft is introduced into a protected mouse, little, perhaps no, proliferation follows, and the tissue speedily dies. Whether it is killed by the fluids of the body, or simply starved to death, because it is prevented from exercising its chemiotactic influence on the connective

tissues, is a problem too hypothetical for discussion here; it will suffice to say, that interference with the chemiotaxis of the cells of tumours of different kind would explain the phenomena thus far observed, their specific and their common features.

Is the protection to be interpreted as due to some virus contained in the cancer cell? I think not. We have pointed out how mere inhibition of the connective tissue reaction would suffice to prevent growth, since its specificity indicates a delicacy of nutritive requirements on the part of the cancer cell far exceeding that of any other known biological reaction. We found that protection is conferred on the mouse only by mouse tumour, not by the preceding inoculation of the tumours of alien animals even so nearly related as the rat. One form of mouse tumour protects against another. We found that protection is conferred on the mouse by the normal tissues of the mouse and not by those of strange species. There is thus something common to mouse tissue and mouse tumour which is not common to mouse tumour and the tumours of other animals. The protection cannot therefore be due to the presence in the tumour cells of a virus common to vertebrate cancer. Reviewing our experiments as a whole, I am of the opinion they establish that the cancer cell is really a cell of the mouse organism requiring the same food, the same kind of connective tissue and blood supply, to which it was accustomed in the mouse in which it developed. The only difference it exhibits is a qualitative one, viz. in the powers of growth and assimilation. Fortunately the cancer cell is very much at the mercy of subtle changes in its environment, and it is by taking advantage of this fact that we have been able to prevent its growth in the living mouse.

The study of cancer in man became increasingly difficult in the course of time because of the mere multiplication of facts defying classification. Malignant new growths in man and in animals—even those of any particular organ and histogenetically related—exhibit an extraordinary variety in their histology and clinical behaviour. This variability is made yet more difficult to comprehend by the inconstancy in the behaviour of tumours histologically indistinguishable. Many attempts have been made to group the phenomena, *e.g.* by pushing the histogenetic subdivision of tumours to absurd lengths, by assuming that progressive loss of histological differentiation proceeds *pari*

passu with prolongation of growth (anaplasia), and, for apparently identical tumours, by assuming differences in the soil afforded by different individuals, and also by the several organs of any single individual. At the outset of our investigations, therefore, the growth of cancer under natural and experimental conditions was selected for study, as covering ground common to all the varied manifestations of tumours. The experimental study of the growth of cancer proved the permanence of the histological characters, and revealed that the cells possessed other qualities of a more or less permanent kind, escaping demonstration by other means. At the same time, experiment shed light on the relations subsisting, on the one hand, between the different properties of cells similar morphologically, and on the other hand the soil in which they are situated. Only by experiment has it become possible to study the growth of one tumour in different soils, and, what is equally important, the growth of different tumours in the same soil. In this way the varying clinical and histological features of the cancers of the mouse's mamma, reproducing as they do in miniature all the difficulties encountered in man, have been relegated to subsidiary importance for the present, in favour of the study of the problem of cell assimilation, which, being common to all these tumours, permits us to unite in one general conception all the gradations and mutations exhibited in the growth of cancer. The experimental study of growth has already enabled us to compare tumours together as regards their biological behaviour (clinical behaviour). It has been proved that cancer grows better in young mice than in old; that some mice of the same age afford a more suitable soil than others; that some sites of the body favour growth while others hinder it; that tumours seemingly identical (when examined by the aid of other methods) can be differentiated from one another, since they possess distinct properties by virtue of which they behave differently in the same soil; that one and the same tumour exhibits increased proliferation alternating with diminished proliferation in a similar nutritive environment, and, this being so, the fluctuations in proliferation are possibly expressions of an alternation in the assimilative powers of the cells of the tumours which exhibit them. Experimental propagation further showed that proliferation can be maintained by normal bipolar division and that other forms of cell division are

unimportant. Division being merely the end phenomenon in the growth of the individual cell, what requires study is not the manner in which the cells divide, but the mechanism of cell assimilation and its relation to nutritional environment. These problems have been attacked experimentally by altering the conditions under which cancer is artificially propagated, and, as I have pointed out above, the nutritional environment can be so altered that the cancer cell is unable to grow. The study of the growth of cancer further permits inferences as to the nature of the changes, in cells and in organisms, leading to the inception of the continuous assimilation of which cancer cells are capable.

Murray and myself pointed out the importance of separately considering two factors in studying the growth of cancer—viz. the conditions of origin and the conditions of growth—and we pointed out that artificial propagation enabled us to study the conditions of growth experimentally. Ehrlich has since penetrated more deeply into the relation of these factors by differentiating the primary cell changes in a circumscribed area from the conditions permitting the cells in such an area to grow into a tumour, and by assuming the importance of constitutional conditions favourable to growth. We have pointed out, with reference to carcinoma mammæ in the mouse, that the differences revealed and shown to be retained in artificial propagation indicate primary differences inherent in the cells of different tumours, and that they can be conceived as due either to a variety of causes or to one causative factor acting in varying degree. As our work proceeded, evidence accumulated pointing more and more definitely to the assumption that one primary change occurring in varying degree explained the different behaviour of apparently similar and nearly related tumours; but we are ignorant of how the primary change is elicited. If histological differences be ignored for the present, the only fundamental differences, from the standpoint of growth, are in the energy of assimilation of the cells, and in the chemiotactic influences they exert on the connective tissues of the host which convey the food supply. In accordance with this conception what are abstractly called variations or differences in malignancy between tumours of the same histological type are concretely expressed as differences in rate of growth. The injury to the mouse in which

a transplanted tumour is growing proceeds only from the consequences of growth, and the demands made for nutriment; but as a rule the mice look quite well, and malnutrition progressing to wasting is an occasional and accidental occurrence.

Thus far I have considered only the growth of cells, but we have to deal with the growth of tumours often weighing nearly as much as a mouse. If cell nutrition is so important it may seem strange to say that the mice usually enjoy perfect health. It was necessary to ascertain if the nutritive balance in an animal had been disturbed by the presence of a tumour in any other way than could be indicated by ill-health and wasting. When very young mice are made to bear rapidly growing and large tumours, they often only attain about half the weight of others of corresponding age. When adult animals bear rapidly growing and large tumours they may lose weight, and more than is accounted for by the weight of the tumour. This they speedily make good when the tumours are removed, and the mice then revert to the normal conditions without any detectable sign of their having ever borne tumours. The gastric contents of normal mice after successful inoculation with tumours contain a large excess of HCl during active digestion, as observed by Cramer and myself, and demonstrated by Copeman and Hake, who have analysed the stomach contents of over five hundred mice. This increase in HCl is not to be considered as out of accord with clinical observations in the human subject. There is much evidence, recently added to by the careful investigations of B. Moore, that the HCl is diminished in patients suffering naturally from cancer. I am at present considering the facts on the growth of cancer in *normal* mice. We require more data regarding mice spontaneously affected with cancer. The contradiction between the state of affairs in normal mice artificially made to bear tumours, and patients bearing them naturally, reinforces the warning I have expressed already. The conditions of the growth of cancer are to be sharply distinguished from the conditions of origin, and I have also pointed out that the conditions of growth in mice naturally cancerous, and in normal mice, differ from one another. The contradiction above referred to is probably only apparent, and when it is resolved we may know much more of the deeper significance of the diminution in HCl in those spontaneously

affected with cancer. This increase in the amount of physiologically active hydrochloric acid is to be regarded as a compensatory response to the needs of a normal mouse plus a tumour. No assumption is made in inferring that the increase in hydrochloric acid is required for the digestion of proteids. The increase in hydrochloric acid is in all probability accompanied by increased secretion of pepsin and trypsin, the ferments concerned in proteid digestion, although this is difficult to determine in mice. The increased activity of proteid digestion is easily comprehended. Whereas, in a general way, the combustion of carbohydrates suffices for the output of energy in an animal organism, proteids are essential to the building up of protoplasm, and the building up of protoplasm is proceeding apace in the continual production of new tumour cells.

On a preceding page I have pointed out the specific characters of the proteids which even nearly allied animals reconstruct from identical pabulum, and how the mouse alone supplies the nutritive environment or pabulum suited to the continued growth of mouse cancer. These facts are to be remembered in the present connection, for there is no need to assume that the increase in proteid digestion involves any other divergence from the normal process. No doubt proteid digestion prepares the food-stuffs for absorption into the fluids of the body in the usual way, and for their utilisation by its constituent cells and by the cells of the tumour. So fine an adjustment can only mean that the cancer cells of the mouse and normal mouse cells compete for the products of proteid digestion. In view of what we know of the specificity of these processes of nutrition, on which so much light has been thrown by Starling and Bayliss, this implies that the cells of mouse cancer are mouse cells able to impress their needs for food on the mouse as a whole by their higher assimilative energy. The facts reveal, at any rate, how great are the demands for food which growing tumours make on healthy and young adult animals, and at the same time show that the normal animal responds to these demands at least by increased proteid digestion. These observations are the mere beginnings of the experimental study of the problems of metabolism in cancer, and which are not easy to attack in so small an animal as a mouse, and will be attacked still more profitably in the case of larger animals, *e.g.* the dog. The problems of excretion which may have practical importance

are not capable of easy solution in the mouse. One can use the organism as an indicator of the nutritive needs and metabolic activity of cells, because in studying propagated cancer we have such a large number of tumour cells of the same kind in one animal. Conversely, as I have shown above, we can use the tumour cells as indicators of changes in the animals. So far as these results go they encourage further experimental investigation into the growth of cancer as a problem of cell nutrition, in which the organism as a whole is implicated, although the demand proceeds from a circumscribed area, limited in the first place to a little bit of tissue no bigger than a pin's head at the time of inoculation. It is obvious that this line of inquiry may have ultimately direct bearings on certain aspects of the disease in man; but I must enforce caution in drawing conclusions, and above all warn the layman that I have said nothing in favour of popular views on diet or disordered digestion as causes of cancer.

This is an opportune stage at which to terminate an outline of the firstfruits of the experimental investigation of cancer, to the development of which the Imperial Cancer Research has very materially contributed. In 1905, 137,128 deaths of males occurred in England and Wales above the age of 35; of these 11,908 were due to cancer. In the same year there were 138,477 deaths of females above 35 years of age, of whom 16,875 died of cancer. Therefore of persons dying above the age of 35, one out of every eleven men died of cancer, and one out of every eight women. Is this great frequency as a cause of death to be explained as due to communication of the disease from one person to another? Our experimental investigations have revealed no analogy with any known form of infection. The chance of ultimately dying of cancer is one in eight for women and one in twelve for men above the age of 35. A simple calculation shows the probabilities of the occurrence of one or several cases of cancer in families of whom few or many survive beyond the age of 35. Murray and myself have pointed out that when the full facts are known, the incidence of cancer in some animals will probably approximate to that in man, although the forms of cancer to which different species are most liable may differ. Since the greater frequency of cancer coincides with the later years of life, the span of life of human beings has long been one of the obstacles preventing a final

settlement of the question whether there is or is not a family liability to the disease. This obstacle is inoperative in the case of the mouse, with a short life of two to three years. Statements have been made of the greater frequency of cancer in the mice housed in certain cages, but no account has been taken of the total population of the cages for the period during which the tumours were observed, nor of the sex and age-constitution of the mouse population. In short, the data necessary to justify statements of relative frequency have not been ascertained. To baldly assert that epidemics occur, or that the disease must have been conveyed from one mouse to another because so and so many cases of cancer have occurred in one cage, as has been done in the most positive manner by the New York State Laboratory, Buffalo, U.S.A., is to ignore the rudiments of statistics. By breeding and in-breeding mice of cancerous stock we hope to finally settle the importance attaching to the apparent frequency of cancer in some strains of mice as compared with others. We have long had such observations in progress; but, since statistical studies on the incidence of cancer in animals require to be conducted with all the precautions accurate statisticians employ in dealing with the incidence of cancer in a human population, we are not yet able to institute comparisons between different communities of mice. These observations do not lend support to assertions of infection, any more than do our studies on the nature of experimental transference, and the means whereby immunity or protection may be conferred. The universality of the disease in man and animals, the biological law of its age incidence, the unique character of the proliferation and its continuation on transplantation, the peculiar nature of the measures which protect mice, as well as the intimate relations between normal and cancerous tissue, and the increased digestive activity induced to compensate for the building up of protoplasm in the tumours borne by otherwise normal animals, all point to the probability that cancer arises *de novo* in the individual attacked. This statement may seem to be mere dogmatic reiteration of an oft-expressed hypothesis; but experiment has removed this conception of the relation of cancer to the individual from the realms of hypothesis to the level of a well-considered theory, harmonising many isolated facts, and fruitful as the basis of further inquiry.

Finally I may point out, that if the comparative and experi-

mental investigation of cancer has revealed the fallacious nature of all reputed analogies with known forms of infection, the results obtained are essentially of constructive value. The cell problems being attacked now were unapproachable five years ago, as were also the relations existing between tumours and the organisms bearing them. Having recognised fully, and insisted from the outset of our investigations, that the reproduction of the lesions of cancer in normal animals by transplantation differed from the development of the disease *ab initio* in animals spontaneously affected, I have confined myself largely to describing the growth of cancer in the soil provided by normal animals, and to drawing some inferences as to the conditions obtaining in the development of cancer. I have shown that even in normal animals the soil plays an important part, and a part which can be modified at will. I have indicated that the soil may play an important part in the spontaneous appearance of cancer in individuals. I have also shown that the primary properties of cancer cells differ. Therefore the circumstances associated with the development of cancer require to be considered with reference to the two sets of factors last mentioned.

The extent to which the experimental method has already deepened our knowledge of the properties of cancer cells and of their relations to normal animals renders it probable, that its further application will throw light on the conditions underlying the association of the inception of cancer with the senescence of tissues, and on the increasing frequency of the disease as age advances, throughout the vertebrate kingdom.